ELSEVIER

Pharmacology, Biochemistry and Behavior 73 (2002) 911–919

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Interactions between the CB1 receptor agonist Δ^9-THC and the CB1 receptor antagonist SR-141716 in rats: Open-field revisited

Torbjörn U.C. Järbe*, Matthew E. Andrzejewski[1], Nicholas V. DiPatrizio

Temple University, Department of Psychology, 265-67 Weiss Hall, 1701 North 13th Street, Philadelphia, PA 19122, USA

Received 7 March 2002; received in revised form 11 June 2002; accepted 26 June 2002

Abstract

This study examined the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) and the CB1 antagonist SR-141716 on open-field behaviors in male Sprague–Dawley rats. Animals were examined after administration of Δ^9-THC alone (dose range: 0.3–5.6 mg/kg), SR-141716 alone (dose range: 1–5.6 mg/kg) and the two drugs in combination; injections were given intraperitoneally 30 min prior to testing. There was a dose-related suppression of ambulation (horizontal activity) and rearing (vertical activity) after Δ^9-THC administration. Co-administration of SR-141716 counteracted this suppression; however, antagonism was only partial for rearing. Interestingly, 1 mg/kg SR-141716 was as effective as 3 and 5.6 mg/kg SR-141716 in this antagonist action. Increasing doses of Δ^9-THC produced an increase in circling behavior; latency to leave the starting area in the center of the field was significantly elevated by 5.6 mg/kg Δ^9-THC. Those effects were completely blocked by SR-141716. Grooming and scratching showed a dose-related increase following administration of SR-141716 (1–5.6 mg/kg), which were only partially blocked by co-administration of Δ^9-THC (3 and 5.6 mg/kg). When given alone, only the highest dose of SR-141716 (5.6 mg/kg) depressed ambulation; rearing and latency were not significantly changed, and circling was absent. Differences in the number of vocalizations, urination and defecation generally did not differ clearly among the treatment conditions. These results may show that SR-141716 is acting as (i) an inverse agonist and/or (ii) that the endogenous cannabinoid system is tonically active under certain conditions.

Keywords: Δ^9-THC; Cannabinoid agonist; SR-141716; Cannabinoid antagonist; Open-field; Rats

1. Introduction

The discovery of an endobinoid neural signaling/transmitter modulatory system has created a renewed interest in the actions of classical cannabinoids such as Δ^9-tetrahydrocannabinol (Δ^9-THC; found in hashish and marijuana) and endogenous constituents such as anandamide—both classes of agents producing biological activity through G-coupled cannabinoid receptor activation. Thus far, two receptors have been discovered. The CB1 receptor is localized predominantly in the CNS (Howlett, 1995), but also found outside the CNS (e.g., in the gut and testis). The CB2 receptor is located within the immune system (Munro et al., 1994). To date, most work has focused on the CB1 signaling system and the initially discovered endogenous ligand for this receptor, anandamide (Devane et al., 1992; Palmer et al., 2000). The compound SR-141716 is thought to antagonize CB1 mediated actions of endogenous, as well as exogenous cannabinoids (Pertwee, 1999). However, there are cannabinoid-induced effects that are not readily antagonized by SR-141716. Such findings suggest that not all cannabinoid effects occur through activation of currently known cannabinoid receptor mechanisms (Breivogel et al., 2001; Di Marzo et al., 1998, 2000; Martin et al., 1999; Mechoulam et al., 1998; Monory et al., 2002).

Although THC's and anandamides have many effects in common, there remain key behavioral differences. For example, higher doses of tricyclic cannabinoids such as Δ^9-THC, Δ^8-THC, CBN (cannabinol) and HU-210 (the levo

* Corresponding author. Tel.: +1-215-204-6977; fax: +1-215-204-5539.

E-mail address: tjarbe@astro.temple.edu (T.U.C. Järbe).

[1] Current address: Departments of Psychiatry and Psychology, University of Wisconsin-Madison, 6001 Research Park Blvd., Madison, WI 53719, USA.

0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(02)00938-3

isomer of the 11-hydroxy dimethylheptyl homologue of Δ^8-THC) evoked circling (rotation behavior) in open-field testing (Ferrari et al., 1999; Järbe and Hiltunen, 1987; Sjödén et al., 1973); this did not occur after (*R*)-methanandamide administration (Järbe et al., 1998). Even though surmountable antagonism occurred between Δ^9-THC and SR-141716 (and to some extent also between (*R*)-methanandamide and SR-141716) with regard to the discriminative stimulus effects in rats, higher doses of the agonists in the presence of SR-141716 resulted in reduced rates of lever pressing, especially so for (*R*)-methanandamide (Järbe et al., 2001). Yet, cross-tolerance for the rate depressant effects has been demonstrated between Δ^9-THC and (*R*)-methanandamide in rats maintained on high doses of Δ^9-THC (Lamb et al., 2000). More recently, we examined the antagonistic effects of SR-141716 (dose range: 0.3–10 mg/kg) on Δ^9-THC and (*R*)-methanandamide in rats maintained on a fixed-ratio (FR-10) schedule of food reinforcement. We observed only very limited antagonism of the Δ^9-THC-induced decreases of lever pressing and no antagonism of the (*R*)-methanandamide-induced decreases in operant responding. Rather, the combinations of SR-141716 and (*R*)-methanandamide produced additive effects, resulting in an even more reduced response output than either drug alone (personal observation).

It has become increasingly clear that SR-141716 can exert its own intrinsic actions. They may be viewed as inverse agonism or that the endobinoid system is tonically activated under certain conditions (Pertwee, 1999; Schlicker and Kathman, 2001). In addition to the possibility that SR-141716 may be acting as an inverse agonist or antagonizing the effects of a tonically active endocannabinoid, it may also affect other neurotransmitter systems (e.g., Darmani and Pandya, 2000). The current study revisited the open-field test and examined more fully the interaction between the CB1 receptor agonist Δ^9-THC and the cannabinoid receptor CB1 antagonist/inverse agonist SR-141716 in rats. The open-field situation generates several exploratory, novelty behaviors sensitive to drug manipulation.

2. Method

2.1. Animals

A total of 210 adult male Sprague–Dawley rats (Taconic Farms, Germantown, NY) between 3.5 and 4.5 months old were used. Rats were individually housed with free access to food and water under a 12-h light/dark cycle (lights on at 7 a.m.). Animals were handled each weekday for 2 weeks prior to testing.

2.2. Treatments

Thirty minutes prior to testing in the open-field arena, rats were given two intraperitoneal injections on either side of the peritoneal midline. Groups of rats ($n = 10$) were given either Δ^9-THC and vehicle (Study 1), SR-141716 and vehicle (Study 2), Δ^9-THC and SR-141716 (Study 3), or vehicle and vehicle (controls in Studies 1–3). In Study 1, doses of Δ^9-THC were 0.3, 0.56, 1, 1.8, 3 or 5.6 mg/kg. In Study 2, the doses of SR-141716 used were 1, 3 or 5.6 mg/kg. In Study 3, doses of Δ^9-THC were either 3 or 5.6 mg/kg, while the same range of doses of SR-141716 used in Study 2 were used in Study 3 (1, 3 and 5.6 mg/kg). The smaller doses of Δ^9-THC in Study 1 were not tested again, because prior research, as well as the results of Study 1 indicated that these doses (0.3, 0.56, 1 and 1.8 mg/kg) would not be likely to induce behavioral measures that differed markedly from one another, or from vehicle. They were included in Study 1 to ensure an adequate range for assessing dose dependent effects.

Treatments (i.e., various combinations of drugs and dosages) were staggered such that about one third of the rats in each condition completed the open-field test before the second round commenced, which was followed by the third, and final round. This precaution was aimed at counterbalancing for the possible influence of length of stay in the vivarium prior to testing. Open-field sessions occurred during the lighted portion of the light/dark cycle. No sessions were run on the first day after holidays or weekends.

2.3. Open-field test apparatus

The open-field arena is a gray painted box ($60 \times 60 \times 45$ cm) with an open top and a white floor divided into 16 squares (15×15 cm) and a circle (19 cm in diameter) marked in the center of the field. The floor was covered with a piece of acrylic, which was cleaned between sessions. This is the same open-field arena as that used to examine open-field behavior after cannabinoid administration in previous studies from our laboratory (Järbe et al., 1998 and references cited therein). A video camera was mounted 1.5 m above the floor of the open-field arena, such that the whole arena was viewable on camera. The entire apparatus was centered in an otherwise empty room measuring 2×2.4 m in the Temple University Department of Psychology vivarium. Lighting was provided by overhead florescent lights and two clips-on incandescent lamps with 40-W bulbs about 2 m above the box floor.

Sessions began by placing the rat in the center circle and ended after 5 min. The entire session was recorded on videotape and scored later.

2.4. Behavioral measures

The behavioral measures recorded were (i) ambulation (the number of squares crossed with all four feet), (ii) rearing (the number of times the rat stood erect on its hind-legs), (iii) latency (the time in seconds to leave the starting area, the circle in the center of the field), (iv) circling (the number of times the animals turned around

its vertical axis, 0.5 point given for each 180° turn; we also noted whether the circling or turning behavior consistently was directed to the left or right and whether it shifted during a single open-field exposure), (v) grooming (the number of cleaning bouts), (vi) scratching (defined according to Darmani and Pandya, 2000, i.e., "A scratching episode produced by a particular hind limb consisted of one or more repetitive scratches with less than 2 s in between. If the interval between consecutive scratches by a particular hind limb was greater than 2 s, the scratches were considered as separate episodes. If the scratches were produced by alternative hind legs, then each scratch was considered as a separate episode"; scratching was not recorded in Study 1), (vii) urination and (viii) defecation (the number of urination spots and fecal boli deposited during the 5-min observation period). Also, the presence and absence of vocalization (squeaking) were noted when the rat was lifted up for placement into the open-field arena ("vocalization before"), as well as when the rat was lifted up for removal from the open-field arena ("vocalization after").

2.5. Drugs

Δ^9-THC, dissolved in ethanol (200 mg/ml), was kindly provided by National Institute on Drug Abuse (NIDA), Bethesda, MD, and stored at −20 °C until used. To prepare suspensions, appropriate amounts of Δ^9-THC were withdrawn, the ethanol evaporated under a stream of nitrogen, the residue then dissolved in a solution of 5% propylene glycol and 3% Tween-80, and stored at −20 °C. Shortly before being used, the solute was diluted with normal (0.9%) saline after the solute had been sonicated for 20–30 min. The amount of Tween-80 was increased to 4% for the highest dose of Δ^9-THC (5.6 mg/kg) examined at the expense of saline. SR-141716, as base ((*N*-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; NIDA), was dissolved in a propylene glycol (5%)/Tween-80 (3%) mixture before being diluted with saline. The drugs were administered intraperitoneally in a volume of 2 ml/kg.

2.6. Statistics

Completely randomized one-way and two-way analyses of variance (ANOVA; Kirk, 1968) were calculated using SigmaStat (version 2.0), run on an IBM 166 MHz PC. Subsequent post-hoc analyses used Tukey's honestly significant difference (HSD) test with α=.05, two-tailed, for the collection of comparisons (Kirk, 1968). In Study 3 (see below), in addition to the two-way ANOVAs, one-way ANOVAs were run where the post-hoc contrasts involved comparisons with the vehicle condition only. To better meet the assumptions of homogeneity of error variances and normality of treatment-level distributions, all data except vocalization were square-root transformed for statistical analysis (Kirk, 1968).

3. Results

3.1. Δ^9-THC alone (Study 1)

Fig. 1 shows the effects of Δ^9-THC (dose range: 0.3–5.6 mg/kg) for ambulation [$F(6,63)=20.58$, $P<.001$], rearing [$F(6,63)=25.41$, $P<.001$], circling [$F(6,63)=17.60$, $P<.001$] and latency [$F(6,63)=7.56$, $P<.001$].

As shown in the top two panels of Fig. 1, Δ^9-THC dose-dependently decreased ambulation (upper left panel) and rearing (upper right panel). Post-hoc group comparisons using Tukey's HSD on ambulation revealed that the vehicle, 0.3 mg/kg and 0.56 mg/kg groups were significantly greater than the 1.8, 3 and 5.6 mg/kg groups, that the l mg/kg was statistically greater than the 3 and 5.6 mg/kg groups and that the 1.8 mg/kg group was greater than the 5.6 mg/kg group. An identical pattern of results for rearing was found with the addition of the 1 mg/kg group being greater than the 1.8 mg/kg group. No treatment increased either ambulation or rearing above vehicle levels.

The bottom two panels of Fig. 1 illustrate that Δ^9-THC dose-dependently increased circling (bottom left panel); the latency to leave the circle was significantly increased by 5.6 mg/kg Δ^9-THC (bottom right panel). A significant increase in circling began to appear with 1.8 mg/kg Δ^9-THC as compared to the vehicle condition (see Fig. 1). Additionally, the two highest doses of Δ^9-THC (3 and 5.6 mg/kg) were significantly different from the rest of the treatment conditions. The degree of circling induced by 1.8 mg/kg Δ^9-THC was also significantly different from that observed with 0.3 mg/kg Δ^9-THC (Tukey's HSD). There was no consistent pattern with regard to the directionality of the circling behavior (turning left and/or right during a session), thus, the means represent total circling activity. With regard to the latency to leave the circled area of the center of the open-field, the 5.6 mg/kg Δ^9-THC condition was significantly different from all other test conditions (Tukey's HSD).

ANOVAs for grooming, fecal boli and urination did not reach statistical significance, as was also the case for vocalization before and vocalization after placement in the open-field arena in study 1 ($P>.05$; not shown).

3.2. SR-141716 alone (Study 2)

Fig. 2 shows the effects of SR-141716 (dose range: 1–5.6 mg/kg) for ambulation [$F(3,36)=2.93$, $P=.047$], rearing [$F(3,36)=0.99$, $P>.05$], grooming [$F(3,36)=6.63$, $P=.001$] and scratching [$F(3,36)=15.85$, $P<.001$]. Latency and circling were nonsignificant ($P>.05$; not shown).

Ambulation was statistically significant between the vehicle condition and the highest dose of SR-141716 (5.6 mg/kg) examined in this study (Fig. 2, top left graph); no other pair-wise contrasts reached statistical significance (Tukey's HSD). ANOVA for rearing was nonsignificant (Fig. 2, top right graph). No animals, including the controls, displayed any circling and the mean latency (±S.E.M.) was

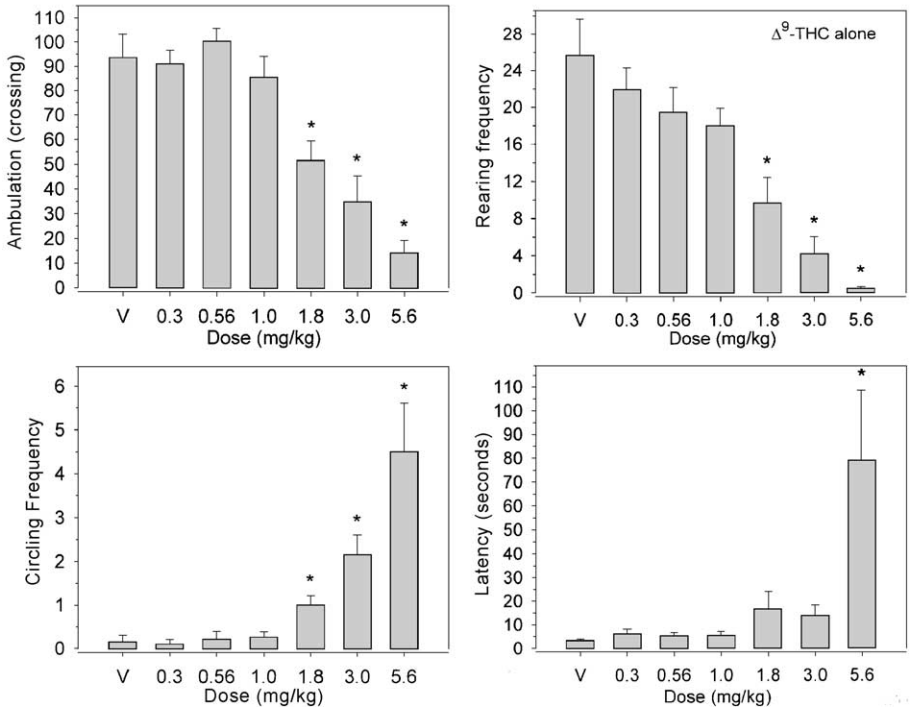

Fig. 1. The effects of Δ^9-THC alone on ambulation (top-left), rearing (top-right), circling (bottom-left) and latency (bottom-right) in different groups of Sprague–Dawley rats ($n = 10$). Δ^9-THC and a vehicle injection (2 ml/kg each) were given intraperitoneally 30 min prior to session onset; controls received two vehicle injections. The data points represent the mean (±S.E.M.) frequency displayed during a 5-min observation period in an open-field arena. * Significantly ($P \leq .05$) different from Δ^9-THC vehicle (V) control; other details in Section 3.

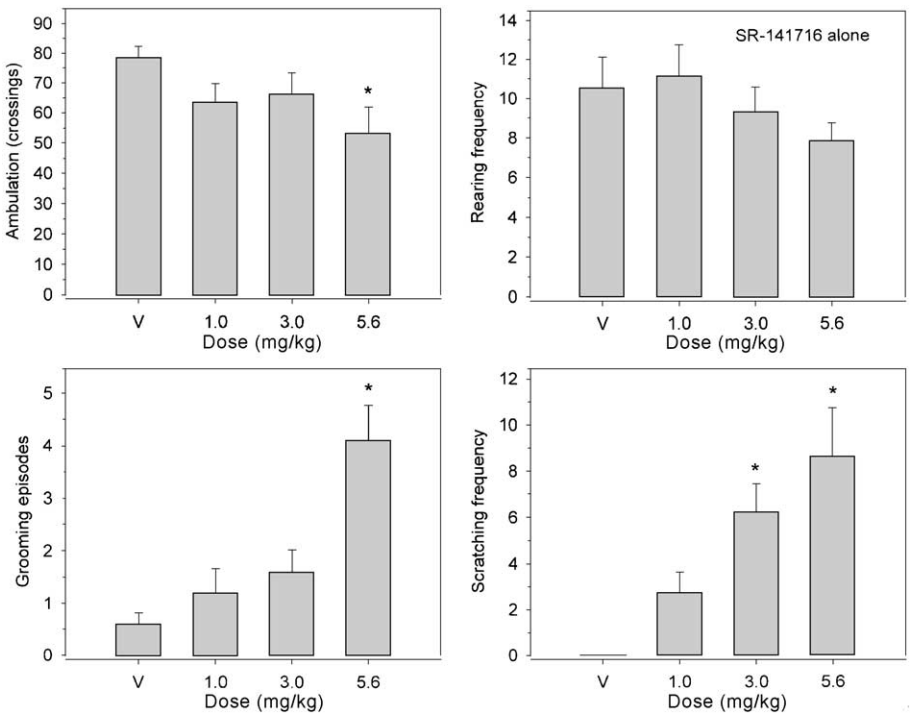

Fig. 2. The effects of SR-141716 alone on ambulation (top-left), rearing (top-right), grooming (bottom-left) and scratching (bottom-right) in different groups of Sprague–Dawley rats ($n = 10$). SR-141716 and a vehicle injection (2 ml/kg each) were given intraperitoneally 30 min prior to session onset; controls received two vehicle injections. The data points represent the mean (±S.E.M.) frequency displayed during a 5-min observation period in an open-field arena. * Significantly ($P \leq .05$) different from SR-141716 vehicle (V) control; other details in Section 3.

2.8 s (0.70) to leave the center circle for the controls; the corresponding values for the 5.6 mg/kg SR-141716 condition was 2.9 s (0.72).

With increasing doses of SR-141716, there were increases in grooming and scratching (Fig. 2, bottom graphs). In addition to grooming being significantly higher than the control condition, 5.6 mg/kg SR-141716 increased the level of grooming significantly above the levels observed with 1 and 3 mg/kg SR-141716 ($P<.05$). Scratching was above control levels for both the 3 and 5.6 mg/kg SR-141716 conditions. Additionally, scratching was also significantly elevated in the contrast between 5.6 and 1 mg/kg SR-141716 (Tukey's HSD).

ANOVAs for fecal boli and urination did not reach statistical significance as was the case also for vocalization before and vocalization after placement in the open-field arena in Study 2 ($P>.05$; not shown).

3.3. Δ^9-THC and SR-141716 in combination (Study 3)

Fig. 3 shows the effects of Δ^9-THC (3 and 5.6 mg/kg) in combination with SR-141716 (0, 1, 3 and 5.6 mg/kg) for ambulation [THC factor, $F(1,72)=26.69$, $P<.001$; SR factor, $F(3,72)=9.50$, $P<.001$], rearing [THC factor, $F(1,72)=8.45$, $P=.005$; SR factor, $F(3,72)=6.23$, $P<.001$], circling [THC factor, $F(1,72)=2.65$, $P>.05$; SR factor, $F(3,72)=11.51$, $P<.001$] and latency [THC factor, $F(1,72)=6.52$, $P=.013$; SR factor, $F(3,72)=7.08$, $P<.001$]. In the case of latency, there was also a significant interaction between the THC and SR factors [$F(3,72)=2.76$, $P=.048$].

Thus, ambulatory activity was markedly suppressed by 5.6 mg/kg Δ^9-THC as compared to the vehicle condition (see Fig. 3, upper left graph), as well as when compared to 3 mg/kg Δ^9-THC. This suppression of ambulation was attenuated by the addition of SR-141716. Note that 1 mg/kg SR-141716 afforded roughly the same degree of antagonism as did 3 and 5.6 mg/kg SR-141716 (Tukey's HSD). A similar pattern of outcome emerged also for rearing. Thus, rearing was suppressed below vehicle levels by both Δ^9-THC doses (see Fig. 3, upper right graph) and rearing was significantly more suppressed by 5.6 as compared to 3 mg/kg Δ^9-THC. Addition of SR-141716 attenuated the Δ^9-THC (5.6 mg/kg)-induced depression of rearing such that all three SR-141716 doses resulted in a level of rearing significantly above the degree of rearing following treatment with 5.6 mg/kg Δ^9-THC alone. However, none of the SR-141716 doses restored rearing to levels comparable to the vehicle condition corresponding to 5.6 mg/kg Δ^9-THC (see Fig. 3). Although the post-hoc analysis following the two-way ANOVA did not indicate a significant increase in rearing with the addition of SR-141716 to 3 mg/kg Δ^9-THC, the lack of statistical significance vs. the corresponding vehicle condition is indicative of a slight restoration of this behavior for the lower Δ^9-THC dose as well. Note that like ambula-

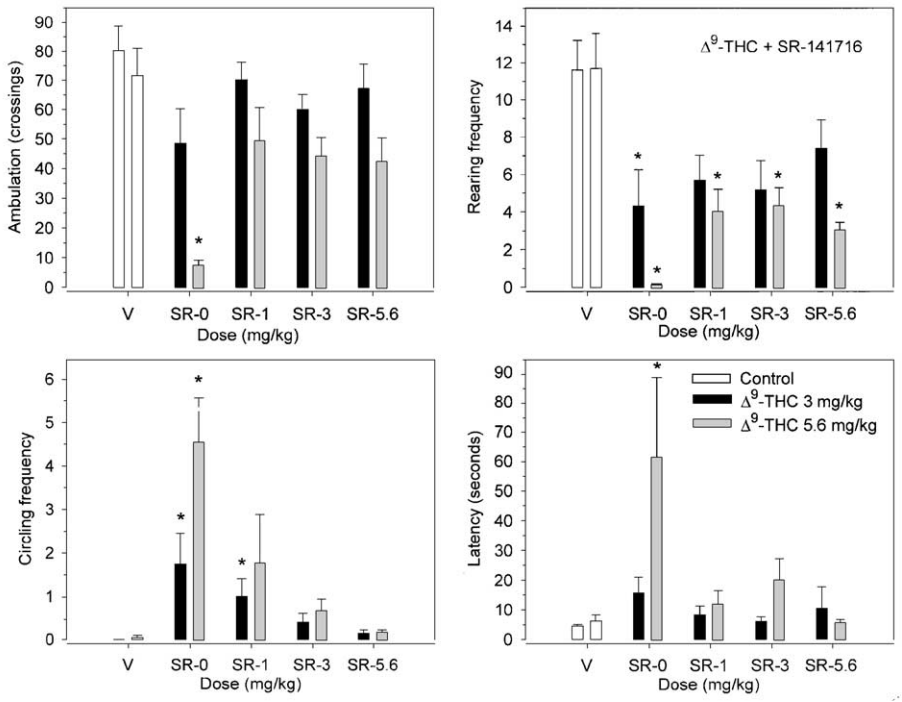

Fig. 3. The effects of Δ^9-THC (3 and 5.6 mg/kg) in combination with SR-141716 (0, 1, 3 and 5.6 mg/kg) on ambulation (top-left), rearing (top-right), circling (bottom-left) and latency (bottom-right) in different groups of Sprague–Dawley rats ($n=10$). Δ^9-THC and SR-141716 (2 ml/kg each) injections were given intraperitoneally 30 min prior to session onset; controls received two vehicle injections. The left white bar above "V" constitutes the control condition pertaining to the interaction study involving 3 mg/kg Δ^9-THC and the right hand white bar refers to the examination involving 5.6 mg/kg Δ^9-THC. The data points represent the mean (±S.E.M.) frequency displayed during a 5-min observation period in an open-field arena. * Significantly ($P\leq.05$) different from Δ^9-THC/SR-141716 vehicle (V) controls (white bars); other details in Section 3.

tion, 1 mg/kg SR-141716 afforded roughly the same degree of antagonism of rearing as did 3 and 5.6 mg/kg SR-141716 (Tukey's HSD).

The degree of circling did not differ significantly between the two Δ^9-THC doses but both doses differed significantly from their respective vehicle condition (see Fig. 3, lower left graph). The addition of SR-141716 dose dependently attenuated the Δ^9-THC-induced circling such that it was nearly totally abolished by 5.6 mg/kg SR-141716 irrespective of the Δ^9-THC dose examined (3 or 5.6 mg/kg). As described in Study 1, directionality of circling was not consistent; therefore the measures represent total (average) circling activity for the 5-min open-field testing period. Latency to leave the circle in the center of the open-field floor was significantly elevated by 5.6 mg/kg Δ^9-THC and that increase was significantly different from all of the other conditions examined ($P<.05$; Tukey's HSD). Addition of SR-141716 markedly attenuated this increase in the latency score (see Fig. 3, lower right graph).

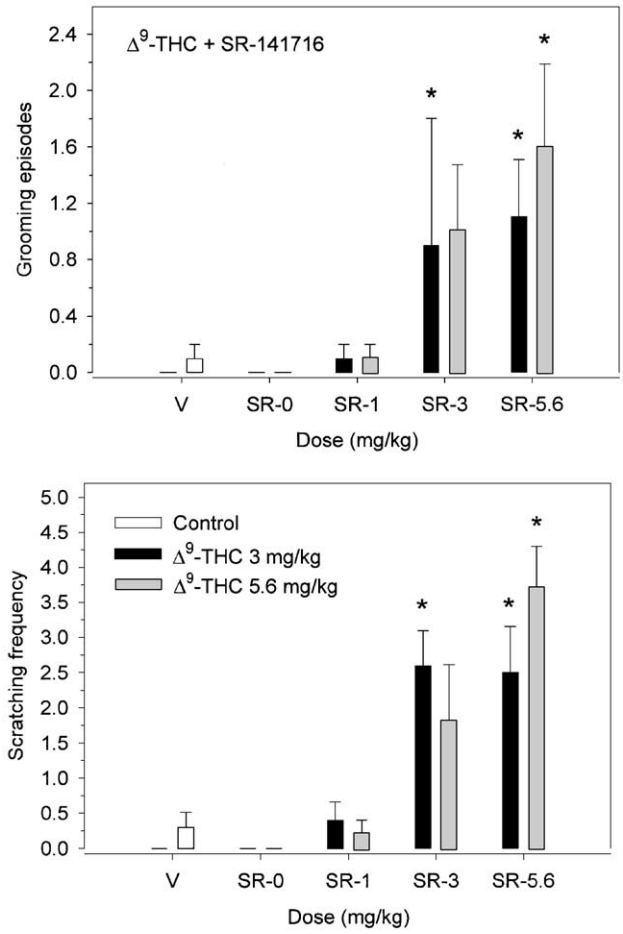

Fig. 4. The effects of Δ^9-THC (3 and 5.6 mg/kg) in combination with SR-141716 (0, 1, 3 and 5.6 mg/kg) on grooming (top) and scratching (bottom) in different groups of Sprague–Dawley rats ($n=10$). Δ^9-THC and SR-141716 (2 ml/kg each) injections were given intraperitoneally 30 min prior to session onset; controls received two vehicle injections. The left white bar above "V" constitutes the control condition pertaining to the interaction study involving 3 mg/kg Δ^9-THC and the right hand white bar refers to the examination involving 5.6 mg/kg Δ^9-THC. The data points represent the mean (±S.E.M.) frequency displayed during a 5-min observation period in an open-field arena. *Significantly ($P\leq.05$) different from Δ^9-THC/SR-141716 vehicle (V) controls (white bars); other details in Section 3.

Fig. 4 shows the effects of Δ^9-THC (3 and 5.6 mg/kg) in combination with SR-141716 (0, 1, 3 and 5.6 mg/kg) for grooming [THC factor, $F(1,72)=0.05$, $P>.05$; SR factor, $F(3,72)=11.96$, $P<.001$] and scratching [THC factor, $F(1,72)=0.08$, $P>.05$; SR factor, $F(3,72)=28.47$, $P<.001$].

Doses of 3 and 5.6 mg/kg SR-141716 (in combination with Δ^9-THC) resulted in an increased number of grooming episodes as compared to vehicle (see Fig. 4, top graph), as well as above the levels associated with administration of Δ^9-THC alone and together with 1 mg/kg SR-141716; there was no significant difference between the two Δ^9-THC doses. The outcome with regard to scratching was very similar to that for grooming. Thus, the two higher doses of SR-141716 increased the number of scratching episodes significantly above vehicle levels (see Fig. 4, bottom graph), as well as above the levels associated with administration of Δ^9-THC alone and together with 1 mg/kg SR-141716 ($P<.05$; Tukey's HSD); there was no statistically significant difference between the two Δ^9-THC doses.

Two-way ANOVA suggested significance for the SR factor with regard to fecal boli [$F(3,72)=3.38$, $P=.023$]. Tukey's HSD isolated a main effect between 3 mg/kg SR-141716 + Δ^9-THC (mean: 0.93) vs. Δ^9-THC (mean: 0.34) alone; urination did not reach statistical significance (not shown). Two-way ANOVA suggested significance also for the SR factor with regard to vocalization occurring after the open-field session in Study 3 [$F(3,72)=4.82$, $P=.004$]. Tukey's HSD isolated main effects between 5.6 mg/kg SR-141716 + Δ^9-THC (mean: 0.30) vs. (i) Δ^9-THC alone (mean: 0.80) and (ii) Δ^9-THC + 1 mg/kg SR-141716 (mean: 0.75).

4. Discussion

In the current study, several doses of the cannabinoid receptor agonist Δ^9-THC and the CB1 receptor antagonist SR-141716 were evaluated singly and in combination using open-field (unconditioned) behaviors of rats. As has been described before, Δ^9-THC singly suppressed ambulation and rearing at higher doses (e.g., Järbe et al., 1998 and references cited therein). At low doses, Δ^9-THC sometimes produces increases in some measures of ambulation and rearing activity; no increases in these parameters were detected in the current investigation in spite of an extended dose range (0.3–5.6 mg/kg) of Δ^9-THC being examined. The degree by which one might detect excitatory effects of Δ^9-THC invariably relates to the control levels in a between-groups comparison design. Other potential factors relate to dosing regimen, e.g., time after drug administration and tolerance development (see, e.g., Drew et al., 1972; Sjödén et al., 1973).

The suppressed ambulatory, horizontal activity was counteracted by co-administration of SR-141716. All three doses of SR-141716 (1, 3 and 5.6 mg/kg) appeared equally effective in this regard. Thus, 1 mg/kg restored ambulation to roughly the same degree as did 5.6 mg/kg SR-141716. The effect of 5.6 mg/kg Δ^9-THC seemed less well normalized by SR-141716 than did the suppression induced by 3 mg/kg Δ^9-THC. This extends data recently described by Arévalo et al. (2001) for rats treated with the bicyclic cannabinoid receptor agonist CP-55,940 and examined in the hole-board and elevated plus-maze tests; only one dose (3 mg/kg) of SR-141716 was examined. A pattern similar to that of ambulation emerged also for rearing in the current study. However, after administration of 5.6 mg/kg Δ^9-THC, none of the three doses of SR-141716 employed were able to fully restore rearing to levels comparable to the corresponding control values. Again, all three doses of SR-141716 behaved much in the same way, producing essentially a flat dose–response curve. A dose of 3 mg/kg SR-141716 failed to restore CP-55,940 suppressed rearing in rats to control values in the hole-board test (Arévalo et al., 2001; see also Darmani, 2001a,b).

Corroborating previous data (Järbe et al., 1998 and references cited therein), there was a dose-related increase in circling or rotation behavior with increasing doses of Δ^9-THC. Given the extended dose range investigated here, the threshold dose for the appearance of this idiosyncratic behavior was determined to be 1.8 mg/kg Δ^9-THC in Study 1. Additionally, no consistent patterns in the direction (i.e., left or right) of the circling appeared. Thus, the same rat may have displayed circling in either direction (or both) during a given open-field session. Circling has also been observed after administration of other classical, tricyclic agonist cannabinoids such as Δ^8-THC (Sjödèn et al., 1973), CBN (Järbe and Hiltunen, 1987), as well as the very potent 11-hydroxy dimethylheptyl homologue of Δ^8-THC (Järbe et al., 1989; Mechoulam et al., 1988), commonly referred to as HU-210 in the literature (Ferrari et al., 1999). Interestingly, not much circling behavior in the open-field test was elicited after treatment with (*R*)-methanandamide (Järbe et al., 1998), a chiral analog of the naturally occurring receptor ligand anandamide (Abadji et al., 1994). In drug discrimination studies; however, (*R*)-methanandamide and Δ^9-THC substituted for each other's discriminative stimulus effects (Järbe et al., 2001). The increased circling observed after 3 and 5.6 mg/kg Δ^9-THC was dose dependently blocked by co-administration of SR-141716. Thus, unlike the flat antagonism curves described above for ambulation and rearing, 1 mg/kg SR-141716 clearly was less effective in reducing the incidence of Δ^9-THC produced circling than was 5.6 mg/kg SR-141716, confirming previous observations to such an effect (Järbe et al., 1998).

SR-141716 was developed as a specific antagonist of the cannabinoid CB1 receptor (Rinaldi-Carmona et al., 1994), blocking a variety of effects induced by various cannabinoid CB1 agonists in animals (Chaperon and Thiébot, 1999; Pertwee, 1999), as well as significantly attenuating many of the effects of smoked marijuana in humans (Huestis et al., 2001). However, several previous studies have clearly indicated that administration of SR-141716 alone can exert intrinsic activity (Chaperon and Thiébot, 1999; Pertwee, 1999). Thus, SR-141716 may be acting as (i) an inverse agonist and/or (ii) that the endogenous cannabinoid system could be tonically active under certain conditions (Izzo et al., 2001; Schlicker and Kathman, 2001). The current study also examined SR-141716 when administered alone. Over the dose range examined, only a slight decrease in ambulation was observed at the highest dose of SR-141716 tested. There were no statistically significant changes in rearing or in the latency to leave the center circle in the open-field arena; circling was absent. Our pattern of results (see also Arévalo et al., 2001, examining 3 mg/kg SR-141716) contrasts with those of Costa and Colleoni (1999) who stated that "SR-141716 induces in rats a behavioral pattern opposite to that of CB1 receptor agonists." The latter authors reported marked increases in ambulation and rearing after treatment with 3 mg/kg SR-141716. We see no apparent reconciliation of these divergent outcomes in rats except to caution against the use of single-dose experimental designs. More generally, though the direct effects of SR-141716 on motor behaviors are not equivocal and seem to depend on the route of administration, species used and motor behaviors examined (e.g., Compton et al., 1996; Gallate and McGregor, 1999; Masserano et al., 1999; Navarro et al., 1997; Poncelat et al., 1999).

Treatments with SR-141716 were associated with dose-related increases in the frequency of grooming as well as scratching. These increased frequencies of grooming and scratching seemed only partially dampened by co-administration of Δ^9-THC. In mice, Janoyan et al. (2002) observed that high doses of the potent cannabinoid agonists WIN 55,212-2, CP55940 and HU-210 essentially afforded a complete blockade of the scratching induced by 2.5 mg/kg SR-141716 in a dose-dependent fashion. The naturally occurring cannabinoid agonists Δ^8-THC and Δ^9-THC (highest doses tested were 20 mg/kg, respectively) also afforded protection against the SR-141716-induced scratching but the degree of blockade appeared less than that for the above mentioned synthetic cannabinoid agonists. On the basis of pharmacological studies involving relatively site selective antagonists, Darmani and Pandya (2000) concluded that the SR-141716-induced scratching in mice seem to "involve indirect potentiation of serotonergic, glutamatergic and tachykinin neurotransmitter systems." Unlike the current studies and those of Arévalo et al. (2001) as well as Costa and Colleoni (1999) (see also Costa et al., 1999), there was no significant change in the grooming score compared to controls after SR-141716 treatment in the study employing mice (Darmani and Pandya, 2000).

Final notes concern the categories fecal boli, urination and vocalization. A main effect on fecal boli was observed only in Study 3 (interaction between Δ^9-THC and SR-

141716). The general trend though was a reduction in the number of fecal boli deposited as the dose of Δ^9-THC increased as compared to controls in both Studies 1 and 3. SR-141716 tended to counteract that Δ^9-THC related effect. However, in no case were the average levels higher than those for the controls, not even when SR-141716 (1, 3 and 5.6 mg/kg) was examined singly. This contrasts with the finding by Costa and Colleoni (1999), who reported that treatment with 3 mg/kg SR-141716 alone significantly increased fecal boli output above control levels. That agents like these potentially could affect the gastrointestinal tract is supported by data indicating that the gut contains the cannabinoid CB1 receptor (Pertwee et al., 1996; Pertwee, 2001; Izzo et al., 2001). Urination did not differentiate among the treatment conditions. However, before a general consensus can be reached on the significance of such findings, it is probably necessary to better control for food-and water intake and circadian rhythms.

Vocalization (squeaking) only differentiated among conditions in Study 3 where SR-141716 decreased the vocalization induced by Δ^9-THC (5.6 mg/kg) when taking the rats out of the open-field arena. The overall incidence of vocalization in the current studies was less than what we reported before for Δ^9-THC and Δ^8-THC (Henriksson and Järbe, 1972; Järbe and Henriksson, 1973), where we palpitated the rats several times rather than just lifting the animal out of the open-field arena, as done in the current study. Vocalizations have been noted also after treatments with HU-210 (Ferrari et al., 1999) and CBN (Järbe and Hiltunen, 1987), but not significantly different compared to controls after treatment with the nonpsychotropic cannabinoid cannabidiol (CBD; Hiltunen et al., 1988).

These studies indicate that SR-141716 counteracts changes produced by Δ^9-THC administration on exploratory, novelty reactions examined in an open-field arena which is in keeping with previous findings. However, it is also clear that the degree of antagonism achieved with SR-141716 is dependent upon the Δ^9-THC dose and the particular behavior in question. Δ^9-THC-induced circling was monotonically diminished with increasing doses of SR-141716 whereas ambulation and rearing were not. In the latter cases, 1 mg/kg SR-141716 afforded roughly the same degree of antagonism as did 5.6 mg/kg SR-141716. Although the degree of rearing was significantly elevated above that induced by 5.6 mg/kg Δ^9-THC alone, none of the three doses of SR-141716 examined together with 5.6 mg/kg Δ^9-THC restored rearing to levels comparable to that seen after vehicle treatment. It is of course possible that using doses higher than 5.6 mg/kg SR-141716 might have achieved such an endpoint. Although information is limited, in our hands 10 mg/kg SR-141716 together with Δ^9-THC resulted in more depressed lever pressing for food than did 1 and 3 mg/kg SR-141716 in combination with Δ^9-THC (personal observation; see also Järbe et al., 2001). However, the intrinsic activity of SR-141716 described here and elsewhere complicates interpretation. We found that the dose related increase in grooming and scratching after treatment with SR-141716 singly were not markedly counteracted by co-administration of Δ^9-THC. Darmani and Pandya (2000) have suggested the involvement of non-endobinoid transmittor systems for scratching as described earlier (grooming was not changed in the mice study). Thus, one interpretation of our data would be that SR-141716 is acting as (i) an inverse agonist and/or (ii) that the endogenous cannabinoid system is tonically active under certain conditions.

Acknowledgements

We thank the National Institute on Drug Abuse (NIDA), Bethesda, MD for free supplies of Δ^9-THC and SR-141716, and M. Harris for technical support. We also thank two anonymous reviewers for thoughtful comments on an earlier draft of this paper. The Animal Care and Use Committee of Temple University, Philadelphia, PA approved all procedures. The ‘‘Principles of Animal Laboratory Care’’ (NIH publication No. 85-23, revised 1985) were followed. Preliminary results of this investigation were presented at the College on Problems of Drug Dependence (CPDD) 64th Annual Scientific Meeting, Quebec City, Canada, June 8–13, 2002. Supported by NIDA grants DA 09064 and DA 00253.

References

Abadji V, Lin S, Taha G, Griffin G, Stevenson LA, Pertwee RG, Makriyannis A. (*R*)-Methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. J Med Chem 1994;37:1889–93.

Arévalo C, de Miguel R, Hernández-Tristán R. Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. Pharmacol, Biochem Behav 2001;70:123–31.

Breivogel CS, Griffin G, Di Marzo V, Martin BR. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. Mol Pharmacol 2001;60:155–63.

Chaperon F, Thiébot M-H. Behavioral effects of cannabinoid agents in animals. Crit Rev Neurobiol 1999;13:243–81.

Compton DR, Aceto MD, Lowe J, Martin BR. In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta-9-tetrahydrocannabinol-induced responses and apparent agonist activity. J Pharmacol Exp Ther 1996;277:586–94.

Costa B, Colleoni M. SR141716 induces in rats a behavioral pattern opposite to that of CB1 receptor agonists. Acta Pharmacol Sin 1999;20:1103–8.

Costa B, Vailati S, Colleoni M. SR 141716A, a cannabinoid receptor antagonist, reverses the behavioural effects of anandamide-treated rats. Behav Pharmacol 1999;10:327–31.

Darmani NA. Delta-9-tetrahydrocannabinol differentially suppresses cisplatin-induced emesis and indices of motor function via cannabinoid CB(1) receptors in the least shrew. Pharmacol, Biochem Behav 2001a;69:239–49.

Darmani NA. The cannabinoid CB1 receptor antagonist SR 141716A reverses the antiemetic and motor depressant actions of WIN 55, 212-2. Eur J Pharmacol 2001b;430:49–58.

Darmani NA, Pandya DK. Involvement of other neurotransmitters in be-

haviors induced by the cannabinoid CB1 receptor antagonist SR 141716A in naïve mice. J Neural Transm 2000;107:931–45.

Devane WA, Hanûs L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992;258:1946–9.

Di Marzo V, Melck D, Bisogno T, De Petrocellis L. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulary action. Trends Neurosci 1998;21:521–8.

Di Marzo V, Breivogel CS, Tao Q, Bridgen DT, Razdan RK, Zimmer AM, Zimmer A, Martin BR. Levels, metabolism, and pharmacological activity of anandamide in CB1 cannabinoid receptor knockout mice: evidence for non-CB1, non-CB2 receptor-mediated actions of anandamide in mouse brain. J Neurochem 2000;75:2434–44.

Drew WG, Miller LL, Wikler A. Effect of Δ^9-THC on the open-field activity of the rat. Psychopharmacologia (Berlin) 1972;23:289–99.

Ferrari F, Ottani A, Giuliani D. Cannabinoid activity in rats and pigeons of HU 210, a potent anti-emetic drug. Pharmacol, Biochem Behav 1999; 62:75–80.

Gallate JE, McGregor IS. The motivation for beer in rats: effects of ritansirin, naloxone and SR 141716. Psychopharmacology 1999;142:302–8.

Henriksson BG, Järbe T. Cannabis-induced vocalization in the rat. J Pharm Pharmacol 1972;23:457–8.

Hiltunen AJ, Järbe TUC, Wängdahl K. Cannabinol and cannabidiol in combination: temperature, open-field activity, and vocalization. Pharmacol, Biochem Behav 1988;30:675–8.

Howlett AC. Pharmacology of cannabinoid receptors. Ann Rev Pharmacol Toxicol 1995;35:607–34.

Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET, Frank RA. Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. Arch Gen Psychiatry 2001;58:322–8.

Izzo AA, Mascolo N, Capasso F. The gastrointestinal pharmacology of cannabinoids. Curr Opin Pharmacol 2001;11:597–603.

Janoyan JJ, Crim JL, Darmani NA. Reversal of SR 141716A-induced head-twitch and ear-scratch responses in mice by Δ^9-THC and other cannabinoids. Pharmacol, Biochem Behav 2002;71:155–62.

Järbe TUC, Henriksson BG. Vocalization: a characteristic cannabis-induced behavior in the rat? Physiol Psychol 1973;1:351–4.

Järbe TUC, Hiltunen AJ. Cannabimimetic activity of cannabinol in rats and pigeons. Neuropharmacology 1987;26:219–28.

Järbe TUC, Hiltunen AJ, Mechoulam R. Stereospecificity of the discriminative stimulus functions of the dimethylheptyl homologs of 11-OH-delta-8-tetrahydrocannabinol in rats and pigeons. J Pharmacol Exp Ther 1989;250:1000–5.

Järbe TUC, Sheppard R, Lamb RJ, Makriyannis A, Lin S, Goutopoulos A. Effects of delta-9-THC and (*R*)-methanandamide on open-field behaviors in rats. Behav Pharmacol 1998;9:169–74.

Järbe TUC, Lamb RJ, Lin S, Makriyannis A. (*R*)-Methanandamide and Δ^9-THC as discriminative stimuli in rats: tests with the cannabinoid antagonist SR-141716 and the endogenous ligand anandamide. Psychopharmacology 2001;156:369–80.

Kirk RE. Experimental design: procedures for the behavioral sciences. Belmont (CA): Brooks/Cole, 1968.

Lamb RJ, Järbe TUC, Makriyannis A, Lin S, Goutopoulos A. Effects of Δ^9-tetrahydrocannabinol, (*R*)-methanandamide, SR 141716, and *d*-amphetamine before and during daily Δ^9-tetrahydrocannabinol dosing. Eur J Pharmacol 2000;398:251–8.

Martin BR, Mechoulam R, Razdan RK. Discovery and characterization of endogenous cannabinoids. Life Sci 1999;65:573–95.

Masserano JM, Karoum F, Wyatt RJ. SR 141716A, a cannabinoid receptor antagonist, potentiates the locomotor stimulant effects of amphetamine and apomorphine. Behav Pharmacol 1999;10:429–32.

Mechoulam R, Lander N, Feigenbaum JJ, Segal M, Järbe TUC, Hiltunen AJ, Consroe P. Enantiomeric cannabinoids: stereospecificity of psychotropic activity. Experientia 1988;44:762–4.

Mechoulam R, Fride E, Di Marzo V. Endocannabinoids. Eur J Pharmacol 1998;359:1–18.

Monory K, Tzavara ET, Leximе J, Ledent C, Parmentier M, Borsodi A, Hanoune J. Novel, not adenylyl cyclase-coupled cannabinoid binding site in cerebellum of mice. Biochem Biophys Res Commun 2002; 292:231–5.

Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1994;365:61–5.

Navarro M, Herandez E, Munoz RM, del Arco I, Villanua MA, Carrera MR, Rodriguez de Fonseca F. Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. NeuroReport 1997;8:491–6.

Palmer SL, Khanolkar AD, Makriyannis A. Natural and synthetic endocannabinoids and their structure–activity relationships. Curr Pharmacol Design 2000;6:1381–97.

Pertwee RG. Pharmacology of cannabinoid receptor ligands. Curr Med Chem 1999;6:635–64.

Pertwee RG. Cannabinoids and the gastrointestinal tract. Gut 2001; 48:859–67.

Pertwee RG, Coutts A, Griffin G, Fernando SR, McCallion D, Stevenson LA. The presence of cannabinoid CB1 receptors of prejunctional neurones of certain isolated tissue preparations: a brief review. Med Sci Monit 1996;2:840–8.

Poncelat M, Barnouin MC, Breliére JC, Le Fur G, Soubrié P. Blockade of cannabinoid (CB1) receptors by SR 141716 selectively antagonizes drug-induced reinstatement of exploratory behaviour in gerbils. Psychopharmacology 1999;144:144–50.

Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Néliat G, Caput D, Ferrara P, Soubrié P, Breliére JC, LeFur G. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 1994;350:240–4.

Schlicker E, Kathman M. Modulation of transmitter release via presynaptic cannabinoid receptor. Trends Pharmacol Sci 2001;22:565–72.

Sjödén PO, Järbe TUC, Henriksson BG. Effects of long-term administration and withdrawal of tetrahydrocannabinols (delta-8-THC and delta-9-THC) on open-field behavior in rats. Pharmacol, Biochem Behav 1973;1:243–9.